Subpart C—Product Standards

§ 429.25 Standards of quality and purity for protamine.

When protamine is dried to constant weight at 100° C., its total nitrogen content is not less than 22.5 percent and not more than 25.5 percent, and its sulfate content, calculated as SO_4, is not less than 16 percent and not more than 19 percent.

§ 429.26 Standards of quality and purity for globin hydrochloride.

The ash content of globin hydrochloride is not more than 0.3 percent; its nitrogen content, calculated to moisture, ash, and hydrochloric acid free basis, is not less than 16.0 percent and not more than 17.5 percent.

Subpart D—Tests and Methods

§ 429.30 Tests and methods of assay.

The following tests and methods of assay are prescribed for the purposes of the regulations in this part 429. (All reagents specified in this section shall be of U.S.P. quality or better.)

(a) *Tests and methods of assay for insulin injection, protamine zinc insulin suspension, globin zinc insulin injection, isophane insulin suspension, insulin zinc suspension, prompt insulin zinc suspension, and extended insulin zinc suspension.* The tests and methods of assay for insulin injection, protamine zinc insulin suspension, globin zinc insulin injection, isophane insulin suspension, insulin zinc suspension, prompt insulin zinc suspension, and extended insulin zinc suspension shall be those set forth therefor in the U.S.P. or N.F., except that alternative test procedures may be employed when such have been authorized by the Commissioner.

(b) [Reserved]

(c) *Isophane ratio.* The isophane ratio shall be expressed as milligrams of protamine per 100 U.S.P. Units of insulin.

(1) *Reagents*—(i) *The stock buffer solution.* Dissolve in water the quantities of metacresol, phenol, glycerin, and disodium phosphate required to make 10 liters of the batch of isophane insulin and dilute to 1,000 milliliters.

(ii) *The insulin solution.* From a sample of the zinc-insulin crystals to be used in making the batch weigh a quantity which contains 10,000 U.S.P. Units of insulin. Dissolve the crystals in 15 milliliters of 0.1 percent hydrochloric acid. The resulting solution must be clear. Add it to 25 milliliters of the stock buffer solution (paragraph (c)(1)(i) of this section). Dilute with water to approximately 200 milliliters. Adjust the pH to 7.2 using hydrochloric acid or sodium hydroxide. The solution must be clear at this stage. If sodium chloride is to be used in preparing the batch add 25 milliliters of 4.2 percent (w/v) sodium chloride solution. Dilute to 250 milliliters with water. The pH must be between 7.1 and 7.4.

(iii) *The protamine solution.* Weigh 500 milligrams of the protamine to be used in making the batch and dissolve in 10 milliliters of the stock buffer solution (paragraph (c)(1)(i) of this section). If sodium chloride is to be used in preparing the batch add 10 milliliters of 4.2 percent (w/v) sodium chloride solution. Dilute with water to approximately 80 milliliters. Adjust the pH to 7.2 using hydrochloric acid or sodium hydroxide. Dilute with water to 100 milliliters. The pH must be between 7.2 and 7.4 and the solution must be clear.

(2) *Conduct of the test.* Measure six 25-milliliter samples of the insulin solution (paragraph (c)(1)(ii) of this section) into six tubes. To the first tube add 0.60 milliliter of the protamine solution (paragraph (c)(1)(iii) of this section), to the second add 0.72 milliliter, to the third add 0.84 milliliter, to the fourth add 0.96 milliliter, to the fifth add 1.08 milliliters, and to the sixth add 1.20 milliliters. Mix the contents of each tube and let stand for at least 30 minutes. Centrifuge. (Do not filter.) From each supernatant fluid remove two 10-milliliter samples, thus creating two series of samples. To each of one series add 1 milliliter of the insulin solution (paragraph (c)(1)(ii) of this section). To each of the other series add 1 milliliter of the protamine solution (paragraph (c)(1)(iii) of this section). Mix each sample and let stand 10 minutes. Measure the turbidity of each sample by means of a photometer or nephelometer. Plot the readings of the two series of samples, using the amount of protamine originally added in milligrams per 100 U.S.P. Units of